AD-A121 876 ACUTE ORAL TOXICITY POTENTIAL OF 4-NITROPHENYL METHKYL PHENYL PHOSPHINATE(U) LETTERMAN ARMY INST OF RESEARCH PRESIDIO OF SAN FRANCISCO CA T P KELLNER ET AL. SEP 82 1/1

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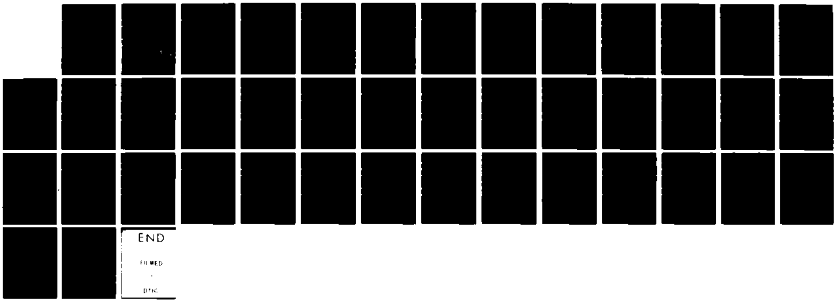

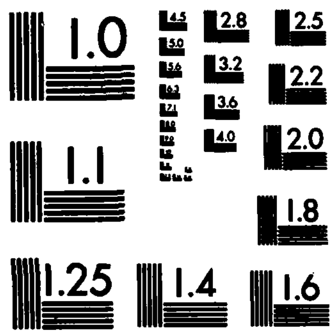
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AD A121876

INSTITUTE REPORT NO. 128

ACUTE ORAL TOXICITY POTENTIAL OF:
4-nitrophenyl methyl phenyl phosphinate

THOMAS P. KELLNER, BS, SP4
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and
JOHN T. FRUIN, DVM, PhD, COL VC

TOXICOLOGY GROUP,
DIVISION OF RESEARCH SUPPORT

SEPTEMBER 1982 **Toxicology Series 35**

LETTERMAN ARMY INSTITUTE OF RESEARCH
PRESIDIO OF SAN FRANCISCO, CALIFORNIA 94129

Acute Oral Toxicity Potential of 4-Nitrophenyl Methyl Phenyl Phosphinate (Toxicology Series 35)--Kellner, Hanes, and Fruin

In conducting the research described in this report, the investigation adhered to the "Guide for the Care and Use of Laboratory Animals," as promulgated by the Committee on Revision of the Guide for Laboratory Animal Facilities and Care, Institute of Laboratory Animal Resources, National Research Council.

John d Marshall Jr 13 Sept 82
(Signature and date)

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7. AUTHOR(s) Thomas P. Kellner, BS, SP4 Martha A. Hanes, DVM, CPT VC John T. Fruin, DVM, PhD, COL VC		8. CONTRACT OR GRANT NUMBER(s) 35162772A875, Toxicity of Phosphinate Compounds WU 304
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19. KEY WORDS (Continue on reverse side if necessary and identify by block number)

Acute Oral; LD_{50}; 4-nitrophenyl methyl phenyl phosphinate; acetylcholinesterase inhibitor; prophylactic agent; organphosphinate

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

The acute oral toxicity potential of 4-nitrophenyl methyl phenyl phosphinate was tested in rats exposed to dose levels ranging from vehicle control (Tween 80, ethanol, citrate buffer and water) to 60 mg/kg body weight. Probit analysis was used to derive values for the median lethal dose LD_{50}. Animals were dosed once and observed for 14 days. The LD_{50} for males was 34.0 mg/kg, with a 95% confidence range of 24.1-48.0 mg/kg. The LD_{50} for females was 12.5 mg/kg, with a 95% confidence range of 7.2-21.6 mg/dk. The toxicity of this material is in the high range. Planned human use experimentation is warranted.

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ABSTRACT

The acute oral toxicity potential of 4-nitrophenyl methyl phenyl phosphinate was tested in rats exposed to dose levels ranging from vehicle control (Tween 80, ethanol, citrate buffer and water) to 60 mg/kg body weight. Probit analysis was used to derive values for the median lethal dose LD_{50}. Animals were dosed once and observed for 14 days. The LD_{50} for males was 34.0 mg/kg, with a 95% confidence interval of 24.1-48.0 mg/kg. The LD_{50} for females was 12.5 mg/kg, with a 95% confidence interval of 7.2-21.6 mg/kg. The toxicity of this chemical is in the high range. Human use experimentation, as planned, is warranted.

PREFACE

Acute Oral Toxicity GLP Study Report

TESTING FACILITY: Letterman Army Institute of Research
Presidio of San Francisco, CA 94129

SPONSOR: US Army Institute of Medical Research and Chemical Defense
Aberdeen Proving Ground, MD 21010

PROJECT: 35162772A875 Defense Against Chemical Agents,
WU 304 Toxicity Testing of Phosphinate Compounds,
APC TL04

GLP STUDY NUMBER: 81033

STUDY DIRECTOR: COL John T. Fruin, DVM, PhD, VC, Diplomate of
American College of Veterinary Preventive Medicine

PRINCIPAL INVESTIGATOR: CPT Martha A. Hanes, DVM, VC
SP4 Thomas P. Kellner, BS

PATHOLOGIST: LTC Paul W. Mellick, DVM, PhD, VC
Diplomate of American College of Pathologists

REPORT AND DATA MANAGER: Carolyn M. Lewis, MS

REPORT AND DATA MANAGEMENT: A copy of the final report, study protocol, and retired SOPs will be LAIR retained in the LAIR Archives.

TEST SUBSTANCE: 4-nitrophenyl methyl phenyl phosphinate

INCLUSIVE STUDY DATES: 25 November - 17 December 1981

OBJECTIVE: To determine the acute oral toxicity potential of 4-nitrophenyl methyl phenyl phosphinate

ACKNOWLEDGMENTS

The authors wish to thank SSG Lance White, SP5 Joe Alletto, BS; and SP4 Evelyn Zimmerman for dosing and clinical observations. The authors also thank Paul Waring, BS for assistance in chemical preparation and analysis.

SIGNATURES OF PRINCIPAL SCIENTISTS AND MANAGERS INVOLVED IN THE STUDY:

We, the undersigned, believe the study number 81033 described in this report to be scientifically sound and the results in this report and interpretation to be valid. The study was conducted to comply, to the best of our ability, with the Good Laboratory Practice Regulations for Medical Laboratory Studies, outlined by the Food and Drug Administration.

John T. Fruin 11 Aug 82
JOHN T. FRUIN / DATE
COL, VC
Study Director

Martha A. Hanes 5 Aug 82
MARTHA A. HANES / DATE
CPT, VC
Principal Investigator

Paul W. Mellick 5 Aug 82
PAUL W. MELLICK / DATE
LTC, VC
Pathologist

Thomas P. Kellner 5 Aug 82
THOMAS P. KELLNER, BS / DATE
SP4
Co-Principal Investigator

Carolyn M. Lewis 5 Aug 82
CAROLYN M. LEWIS, MS / DATE
DAC
Data Manager

DEPARTMENT OF THE ARMY
LETTERMAN ARMY INSTITUTE OF RESEARCH
PRESIDIO OF SAN FRANCISCO, CALIFORNIA 94129

REPLY TO
ATTENTION OF:

SGRD-ULZ-QA 6 Aug 82

MEMORANDUM FOR RECORD

SUBJECT: Report of GLP Compliance

I hereby certify that in relation to LAIR GLP study 81033 the following inspections were made:

25 Nov 81
30 Nov 81
2 Dec 81
3 Dec 81 - 0900 hrs
3 Dec 81 - 1245 hrs
4 Dec 81
17 Dec 81

The report and raw data for this study were audited on 15 Jul 82.

Routine inspections with no adverse findings are reported quarterly, thus these inspections are also included in the Jan 82 report to management and the Study Director.

John C. Johnson

JOHN C. JOHNSON
CPT, MS
Quality Assurance Officer

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Currently the use of organophosphinates as prophylactic agents in anticholinesterase poisoning is being investigated. This research was prompted by the disadvantages incurred with the traditional carbamate prophylaxis. Carbamates were toxic, and the carbamylated cholinesterases did not respond to current oxime therapy. The phosphinates have been shown to be less toxic and chloinesterases repressed by them are believed to more responsive to oximes (Lieske, C.N. et al., in presentation at 181st national meeting of American Chemical Society, Atlanta, GA, March 1981). Combined prophylatic/therapy experiments, using mice, have shown that phosphinate prophylaxis is well founded (Lieske, 1980).

Toxicology Testing

Because of their potential wide-spread use, toxiology testing has been performed or is planned for a number of the organophosphinates. One of these, 4-nitrophenyl methyl phenyl phosphinate was tested by this laboratory for acute oral lethal dose (LD_{50}) in male and female rats. The results of this acute study are outlined in this report.

Objective of the Study

The objective of this study was to determine the acute oral toxicity potential of 4-nitrophenyl methyl phenyl phosphinate.

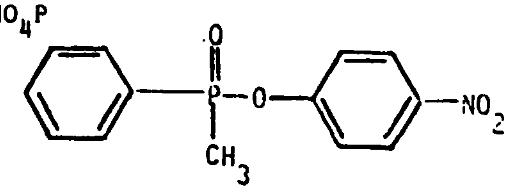
MATERIALS AND CONDITIONS

Chemical Data

1. Chemical Name: 4-nitrophenyl methyl phenyl phosphinate

   Chemical Abstract Service Registry No.: None

   Molecular structure: $C_{13}H_{12}NO_4P$



Molecular weight: 277.2

pH: N/A

Physical state/color: White, fluffy crystals

Melting point: 85-86 C

Compound density: Unknown

Stabiliity: Unknown

Contaminants: None detected. Spectrum analysis attached in Appendix A.

Manufacturer: Ash Stevens Inc. 5861 John C. Lodge Freeway Detroit, Michigan 48202

Manufacturer Lot No: MP-07-29, 5 October 1981

Analysis (by manufacturer):

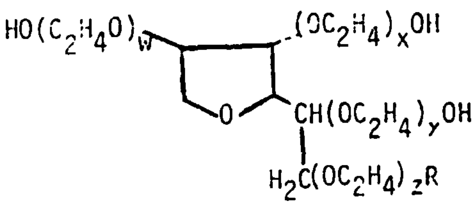
Calculated for $C_{13}H_{12}NO_4P$

Calculated Percent by Weight		Found
C	56.33	56.17
H	4.36	4.28
N	5.05	5.14
P	11.17	11.25

2. Chemical Name: Polysorbate 80 (Tween 80)

Chemical Abstract Service Registry No.: 9005-65-6

Molecular structure:



(Sum of w,x,y,z is 20; R is $(C_{17}H_{33})COO$)

Physical state/color: Liquid, viscous; amber-colored

Stability: Stable at room temperature

Manufacturer: Fisher Scientific Co.,
Fairlawn New Jersey 07410

Manufacturer Lot No: 713137

Published Toxicity Data:

Considered to be pharmacologically inert and is commonly used for dispersing insoluble drugs for oral administration particularly in chronic toxicity studies in experimental data.

3. Chemical Name: Citric Acid, monohydrate

Chemical Abstract Service Registry No.: 77-92-9

Molecular structure:

$$\begin{array}{c} CH_2COOH \\ | \\ HOCCOOH \\ | \\ CH_2COOH \end{array}$$

Molecular weight: 192.12

pH: 0.1N solution = 2.2

Physical state/color: Brachydomatic crystals; white

Melting point: Softens at 75 C and melts at 100 C

Compound Specific Gravity: of aqueous solution, 50% = 1.2204

Stability: Stable at room temperatures

Purity: Contaminents less than 0.3%.

Manufacturer: J.T. Baker Chemical Co.
Phillipsburg, New Jersey 08865

Manufacturer Lot Number: 35444

Published Toxicity Data:

LD_{50} in rats (I.P. Admin.) 975 mg/kg.

4. Chemical Name: Sodium Citrate

Chemical Abstract Service Registry No.: None

Molecular structure:

```
   CH2COO-Na+
   |
HOCCOO-Na+
   |
   CH2COO-Na+
```

Molecular weight: 258

pH: 5% aqueous solution at 25 C = 8.8

Physical state/color/odor: Dihydrate, white, odorless crystals

Melting point: Unknown

Compound density: Unknown

Stability: Stable in air at room temperature; becomes anhydrous at 150 C

Contaminants: less than 0.3% contaminents.

Manufacturer: J.T. Baker Chemical Co.
Phillipsburg, New Jersey 08865

Manufacturer Lot No: 31482

Published Toxicity Data: LD_{50} in rats (I.P. administation) is 6.0 moles/kg (1.5 g/kg).

5. Chemical Name: Ethanol, anhydrous

Chemical Abstract Service Registry No.: 64-17-5

Molecular structure:

$$CH_3CH_2 — OH$$

Molecular weight: 46

pH: N/A

Physical state/odor: clear, colorless, very mobile, flammable, liquid; pleasant odor; burning taste.

Boiling point: 78.5 C

Compound Specific Gravity: 1.361

Stability: Stable if stored properly in a sealed container at room temperature. Will absorb water rapidly from air if not stored properly.

Contaminants: Unknown

Manufacturer: U.S. Industrial Chemicals
Tuscola, Illnois 61953

Manufacturer Lot No: 205

Published Toxicity Data: LD_{50} in rats = 13.7 g/kg

Animal Data

Species: Rat (*Rattus rattus*)

Strain: Sprague Dawley

Source: Charles River
Willmington, MA 01887

Sex: Male and Female

Age: Males - 6 weeks at receipt
Females - 8 weeks at receipt

Method of Randomization: TOXSYS^R Animal Allocation Program

Number of Animals on Test: 6 groups, 7 males and 7 females per group.

Condition of Animals at Start of Study: Normal

Body Weight Range: Males, 114-165 at receipt, 167-206 at dosing
Females, 122-163 at receipt, 149-175 at dosing

Identification Procedures: Ear tag (SOP-OP-ARG-1)

Pretest Conditioning:

a. Quarantine from 25 November - 2 December 1981

b. Animals pre-dosed (acclimated) with 0.5 cc of water daily from 30 November - 2 December 1981

Justification: The Sprague Dawley rat is a proven sensitive mammalian model for oral LD_{50} determination.

Environmental Conditions

Caging: Number/cage = 1; Type cage used = stainless steel, wire mesh bottom, battery type, no bedding.

Diet: Certified Ralston Purina Rodent Diet 5002, Batch No. 81 IE

Water: Central line to cage battery

Temperature: 18 $\pm$ 1 C

Humidity: 65 $\pm$ 15% (Short periods of 80% humidity between 9 and 14 December 1981.)

Photoperiod: 0530 - 2000 hr/day (light, 14 1/2 hr).

Dosing

Dosing Levels

Group 1	Vehicle Control
Group 2	5 mg/kg
Group 3	15 mg/kg
Group 4	30 mg/kg
Group 5	45 mg/kg
Group 6	60 mg/kg

All animals were fasted overnight prior to dosing and received a single dose of the test substance on 3 December 1981. An 18 gauge three inch gastric gavage needle was utilized to administer the chemical by gastric intubation without sedation or anesthesia of the animals.

Actual volume of the test chemical was based on their weight and dose group. The dose was calculated using a Hewlett-Packard calculator system in accordance with LAIR SOP OP-STX-8.

Method of Sample Selection for Chemical Analysis

Samples of the test substance and vehicle were assayed using the spectrophotometric measure of p-nitrophenol for phosphinate determinations (2). These samples were taken before and after dosing on 3 December 1981. Samples were obtained from the same container used in the dosing procedure.

Test Chemical Preparation

Test substances were prepared in accordance with LAIR SOP-OP-STX-48 and details of actual compound preparations are included in the raw data.

Rationale for Selection of the Vehicle

Vegetable oil, used historically in oral LD_{50} studies as the carrier for water insoluble compounds, was not used as the vehicle in this study. This carrier was deemed inadequate for this study because of the tendency of the phosphinate tested to undergo hydrolysis when in solutions of unfavorable pH. A carrier had to be used which would emulsify the compound while stabilizing the pH and allowing for spectrophotmetric monitoring of hydrolysis. A carrier was formulated by the Analytical Chemistry Group (LAIR) which satisfied these requirements (personal communication, P. Waring, Letterman Army Institute of Research, 15 November 1981). This consisted of 21.5% Polysorbate 80 (Tween 80), 37.5% citrate buffer(50 mM, pH 4), 18.5% ethanol and 22.5% water.

Observation Methods and Frequency

Animals were observed daily during the quarantine period. They were observed in and out of their cages and upon replacement for signs of toxicity or altered function during the course of the study at 0730 and 1530 for the first week and at 0730 for the remaining week.

Duration of Study

Animals were quarantined for 7 days and the study continued 14 days after dosing on 3 December 1981.

Historical Listing of Study Events

25 Nov 81	Forty-six male and forty-seven female rats arrived at LAIR. They were sexed, observed for illness, ear tagged, weighed and caged in the GLP suite.
25 Nov 81	One male and one female rat submitted to pathology for quality control at 1450 hours.
30 Nov-2 Dec 81	Rats predosed with 1 ml water.
2 Dec 81	Rats out of quarantine, weighed, observed for illness and randomized into groups. Feed was removed at 1600 hours.
3 Dec 81	Rats were weighed and dosed. Clinical signs were collected during dosing and afterwards.
3-11 Dec 81	Clinical signs were recorded at 0730 and 1530 hours daily. Animals weighed on scheduled days.
12-17 Dec 81	Animals observed for clinical signs at 0730 hours.
16 Dec 81	Feed removed from rats at 1600 hours.
17 Dec 81	Surviving rats were weighed, sacrificed, gross pathological observations performed.

Necropsy Procedures

Animals that died during the day were subjected to necropsy immediately. Animals were not dead for more than twelve hours prior to necropsy. Necropsy was performed in accordance with SOP-OP-STX-32.

Pathology Report

Pathology Report appears in Appendix B.

Statistical Procedures

Animals were randomly assigned to groups using TOXSYS^{R} Animal Allocate System. Groups were randomized using the random program in the User Directory (SOP-OP-ISG-21).

A Fortran V Program on a Data General Eclipse C/330 Computer was used to perform Bliss' method of probit analysis, as described by

Finney (3). The program utilized the percentage kills to determine the weighted regression line of the mortality probit on the log-dose, which results in the formulas:

$$\text{Males } Y = -5.68 + 6.97\ X$$
$$\text{Females } Y = 1.45 + 3.24\ X$$

where Y is the probit and X is the logarithm of the dose. Chi-square statistics were calculated and these values were 3.6 for the male probit and .9 for the female probit. Both lines were statistically acceptable at the chosen level of .05. The probits were then converted back to percentages and the LD_1, LD_{50} and LD_{95} determined along with their 95% confidence limits.

<u>Change in Procedures or Objectives during the Study</u>

Because of the rapid death of many of the test animals, some clinical signs were recorded on sheets other than the official clinical signs/dosing worksheet. This material is included in the raw data. One animal was misdosed in the 15 mg/kg dose group and was removed from the study.

RESULTS

Table 1 shows the number of deaths that occurred in each dose group.

Table 1
Summary of Deaths by Dose Level, Sex and Group

Dose Level	Sex	Death/number
Vehicle Control	male	0/7
	female	0/7
5 mg/kg	male	0/7
	female	1/7
15 mg/kg	male	0/7
	female	3/6 (1 misdose)
30 mg/kg	male	2/7
	female	6/7
45 mg/kg	male	7/7
	female	7/7
60 mg/kg	male	6/7
	female	7/7

Median lethal dose for males and females were graphed inclusive of the 95% confidence interval (Figures 1 and 2, respectively). Table 2 shows LD_1, LD_{50} and LD_{95} with confidence intervals for male and female rats.

Table 2

Lethal Dose (LD) Levels* for Rats Exposed to the Phosphinate

	Males (mg/kg)	Females (mg/kg)
LD_1	16 (3 - 90)	2 (1 - 12)
LD_{50}	34 (24 - 47)	12 (7 - 22)
LD_{95}	59 (23 - 148)	40 (18 - 92)

*with 95% confidence level

The data obtained for male and female rats exposed to 4-nitrophenyl methyl phenyl phosphinate can be compared in Figure 3.

Clinical Observations

Most animals that died are listed on the clinical signs worksheet as dying between 30 minutes and 1 hour after dosing which suggests that if death was to occur, it took place quickly. Under the protocol in use, official observations were to begin 1 hour after dosing was completed. Because of the rapidity of death, the animals were also observed while dosing was in progress. These observations, which are included as a memorandum for record to the raw data, showed that many animals were dead before enough personnel were available for official time of death observations. An example of this was animal D8100735. This male was dosed at 0940 hours (30 mg/kg dose) and was observed dead at 1000 hours. The first official observation which could be recorded on the dosing/clinical signs worksheet was performed at 1030 hours and time of death was recorded as such.

The rapidity of death of many animals explains why one of the most typical clinical signs observed in moribund rats, namely severe body-wide tremors, was not emphasized numerically by the official data summary. Other symptoms that were observed in moribund rats were opisthotonos, foamy salivation, gagging, gasping and lack of muscular coordination. Collapse with severe body-wide tremors and treading was observed in many of the moribund rats in the 60 mg/kg dose group immediately after dosing.

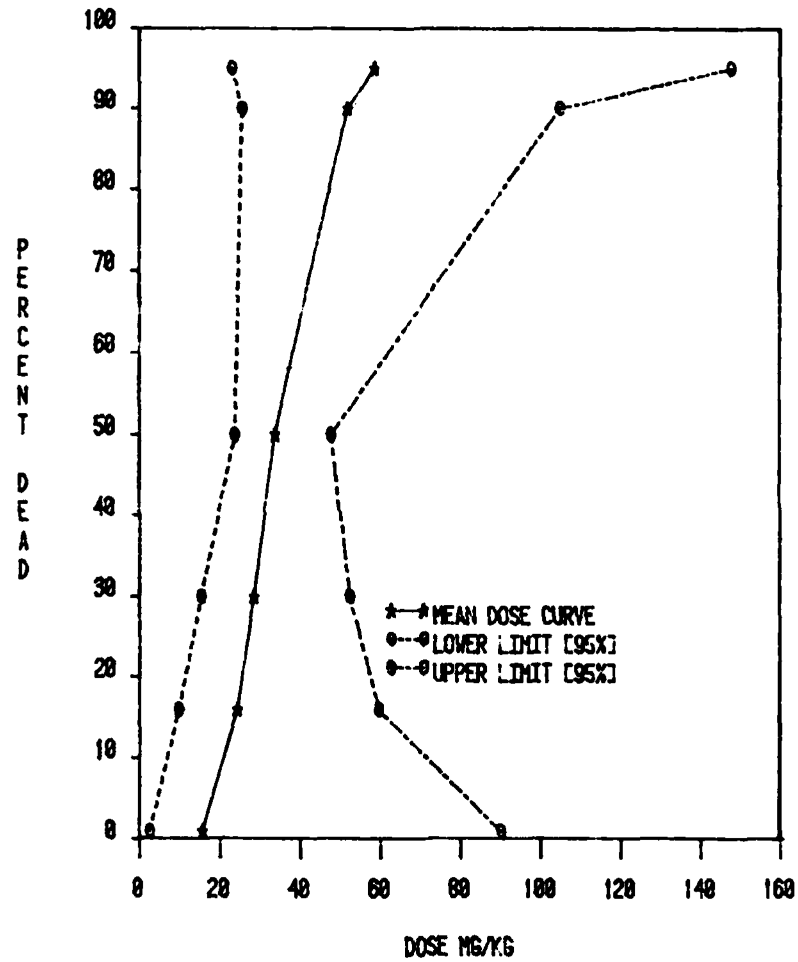


Figure 1. Probit derived dose response curve for male rats with 95% confidence interval.

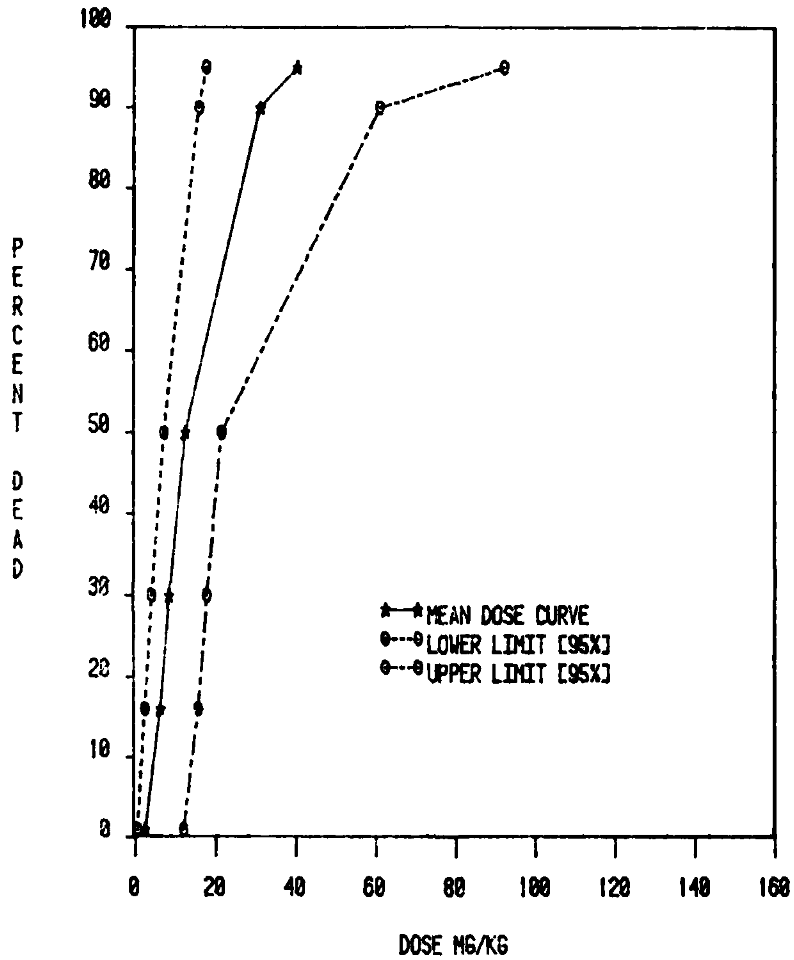


Figure 2. Probit derived dose response curve for female rats with 95% confidence interval.

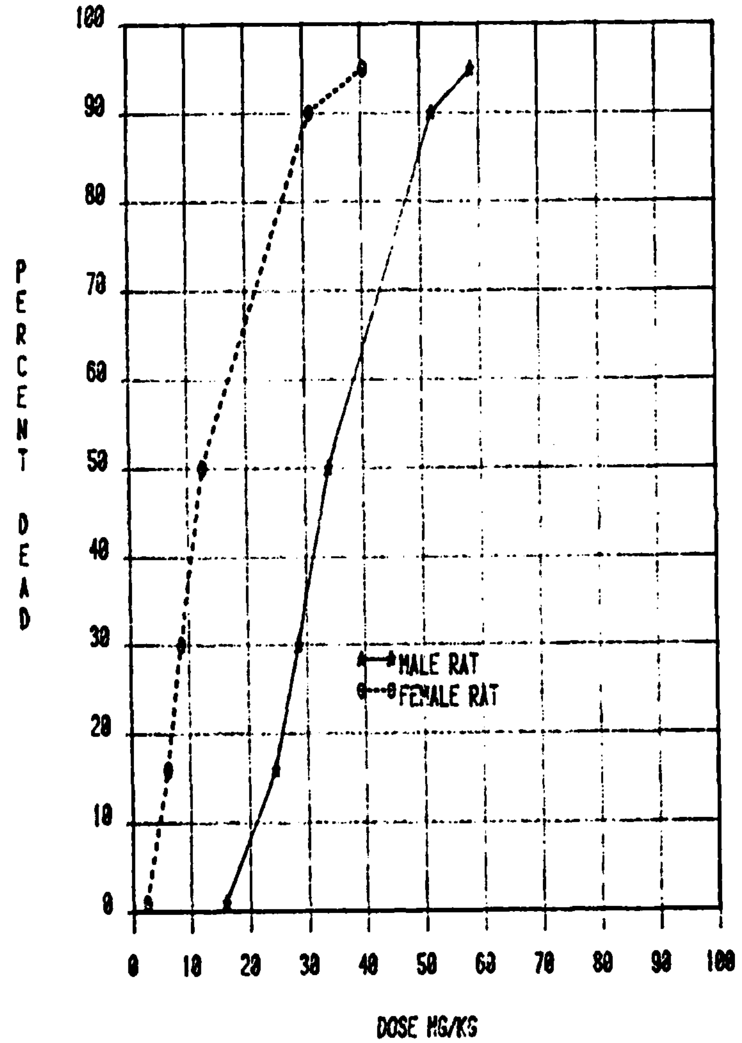


Figure 3. Probit derived dose response curves for male and female rats.

After dosing was completed dead rats were prepared for necropsy while live rats were observed for clinical signs. They were observed in and out of their cages, and upon replacement for signs of toxicity or altered function. The data were recorded by hand on the dosing/clinical signs worksheet and at the termination of the two week observation period the data were summarized by hand.

The summaries contain no female data in the 30, 45 or 60 mg/kg dose group listing because of the high death rates. Only one female (30 mg/kg dose group) survived out of these groups and it was normal in subsequent observations.

The males were less severely effected than the females. Five of seven males survived in the 30 mg/kg dose group as well as one male in the 60 mg/kg group. None survived in the 45 mg/kg dose group.

Clinical Signs - Dose Response

For the males, some clinical signs were reported with increased frequency in response to increased dose levels while others did not show a dose response relationship. Pilo erection and humpback (hunched posture) were among the clinical signs which showed a dose response correlation. Beginning with the vehicle control rats and ending with the 30 mg/kg dose group, the frequency of these signs being reported increased in a regular pattern. The numbers of observations of these signs fell of in the 45 and 60 mg/kg dose groups because of the rapid deaths. Other clinical signs which showed a dose response were tremors, righting reflex, pull reflex, inactivity, yellow material caudal and orange stain cranial.

For the females only two clinical signs showed a recognizable dose response, namely sluggishness and hunched posture. Sluggishness was reported with increased frequency and severity between the vehicle control group and the 15 mg/kg dose group. No signs were recorded for rats in higher dose groups because of the high death rate. Hunched posture increased between the vehicle control and 15 mg/kg dose group in a similar manner.

Clinical Signs - Males

Among the clinical signs most frequently observed in the 5 mg/kg dose group were sluggishness, inactivity and hunched posture. Other signs which were also mentioned were increased respiration rate, panting, rough coat, pilo erection, increased temperature, righting reflex, diarrhea and hair loss.

Hunched posture was the most frequently observed clinical sign in the 15 mg/kg group. Also recorded in more than one animal was rough coat, pilo erection, hair loss and sluggishness. Tremors, righting reflex and inactivity were also mentioned.

In the 30 mg/kg group hunched posture was the most frequently observed symptom. Pilo erection, pinch and righting reflex, inactivity, sluggishness and tremors were also noted frequently.

Others recorded were decreased respiration rate, increased respiration depth, opisthotonos, chewing, rough coat, decreased temperature, staggering, diarrhea and hair loss.

All of the animals in the 45 mg/kg dose group died and so no clinical signs were available for the data summary. Only one rat survived in the 60 mg/kg group. Severe tremors, inactivity and pinch reflex were noted as well as moderately increased respiration rate, increased respiration depth, pilo erection and humpback. Also noted was slight sluggishness, loss of gait and hair loss.

Stains, secretions and other materials were noted in the case of many animals. In the 15 mg/kg dose group, red material in the cranial half of the animal was a common clinical sign (probably the result of harderian secretions). Yellow material caudal and orange stain cranial were frequently mentioned in the 30 mg/kg group.

<u>Clinical Signs - Female</u>

Clinical signs for the females are very scanty because of their low survival rate.

Two of seven rats in the vehicle control group showed slight hunched posture after dosing. For animals in the 5 mg/kg group hunched posture was the most common clinical sign. Pilo erection, sluggishness, pinch reflex and pull reflex were also listed. All of the 3 surviving animals in the 15 mg/kg dose group showed pilo erection and two of three showed moderate tremors, sluggishness and hunched posture. Rough coat, righting reflex, inactivity, staggering, loss of gait and irritability were also mentioned. Clear material in the cranial half of the animal was recorded for animals in this group along with red material cranial and yellow material caudal.

Only one rat survived in the 30 mg/kg group and it was normal as of the first observation period and in subsequent observations. None of the 45 mg/kg or 60 mg/kg group survived to this period and so no data was available for the data summary from these groups.

DISCUSSION

The oral toxicity of 4-nitrophenyl methyl phenyl phosphinate was tested in male and female rats. Signs of intoxication immediately after dosing included severe body-wide tremors, opisthotonos, foamy salivation and lack of muscular coordination. Typical symptoms that persisted beyond the day of dosing included hunched posture, rough coat, pilo erection, inactivity and righting reflex. The LD_{50} values in this study indicate a high toxicity (1-50 mg/kg body weight is considered highly toxic).

Two studies have been performed by this laboratory as a followup to the acute oral LD_{50}. A 14-day subchronic study using 4-nitrophenyl methyl phenyl phosphinate (MPP) was performed to serve as a preliminary test for a 90-day subchronic study and to collect serum, urine and histological data. In addition, the subchronic study yielded data on the effect of prolonged MPP exposure on cholinesterase activity. A 10% depression in red blood cell cholinesterase activity was demonstrated after the rats received sublethal doses of MPP over a 14-day period. No pattern of reduced enzyme activity was seen in brain or plasma from these rats. The second study was an acute oral toxicity assay which involved the dosing of rats with sublethal to lethal doses of MPP. Blood and brain samples were obtained about 1/2 hour after dosing to compare cholinesterase activity with clinical observations made before sacking. The data from this study showed a 70% depression in red blood cell cholinesterase activity in male and female rats approximately 1/2 hour after dosing regardless of dose level. As with the subchronic study, no pattern of reduced enzyme activity was seen in plasma or brain preparations from these animals.

A sex related difference was seen in the levels of normal plasma cholinesterase activity. The females in the control group had 3 to 4 times the plasma cholinesterase activity as the control males. This finding was surprising because females showed a higher sensitivity to the phosphate.

Further investigations into the toxicology of various phosphinate compounds are planned. Work by other laboratories has suggested that peripheral acetylcholinesterase in the diaphragm is the primary lesion in some types of organophosphate poisoning. This avenue can be investigated further by measuring the levels of acetylcholinesterase activity in the diaphragm and other target organs which could be involved in the acute symptoms of organophosphate poisoning.

CONCLUSION

The LD_{50} for 4-nitrophenyl methyl phenyl phosphinate is 34 mg/kg for male rats and 12 mg/kg for female rats.

RECOMMENDATION

Human use experimentation, as planned, is warranted.

REFERENCES

1. PERRIN, D. and B. DEMPSEY. Buffers for pH and Metal Ion Control. New York: John Wiley and Sons, 1974

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APPENDIX A. Chemical Analysis Data

APPENDIX B. Pathology Report

APPENDICES

ASH STEVENS INC.

[illegible]

[illegible]

NOTICE OF SHIPMENT

TO: Commander, U.S. Army Medical Research
Institute of Chemical Defense
Attn: Mr. Claire N. Lieske
Aberdeen Proving Ground
(Edgewood Area) Maryland 21010

YOUR ORDER NO DAMD17-81-1140

DATE SHIPPED October 16, 1981

VIA Federal Express

ITEM	QUANTITY	DESCRIPTION
MP-07-29	30 g	4-Nitrophenyl Methyl(phenyl)phosphinate

PHYSICAL & ANALYTICAL DATA SHEET
AND IR ATTACHED.

Chemical Systems Research and Development

APPENDIX A

ASH STEVENS INC.

DETROIT RESEARCH PARK
5861 JOHN C. LODGE FREEWAY
DETROIT, MICHIGAN 48202
313-872-6400

DAMD17-81-C-1140

PHYSICAL AND ANALYTICAL DATA

Compound: 4-Nitrophenyl Methyl(phenyl)phosphinate

Molecular Weight: 277.2

Melting Point: 85-86°C

Analysis: Calc'd for $C_{13}H_{12}NO_4P$

	Calc'd	Found
C	56.33	56.17
H	4.36	4.28
N	5.05	5.14
P	11.17	11.25

Infrared Data: (Spectrum Attached)

Nuclear Magnetic Resonance Data: ($CDCl_3$, spectrum attached)

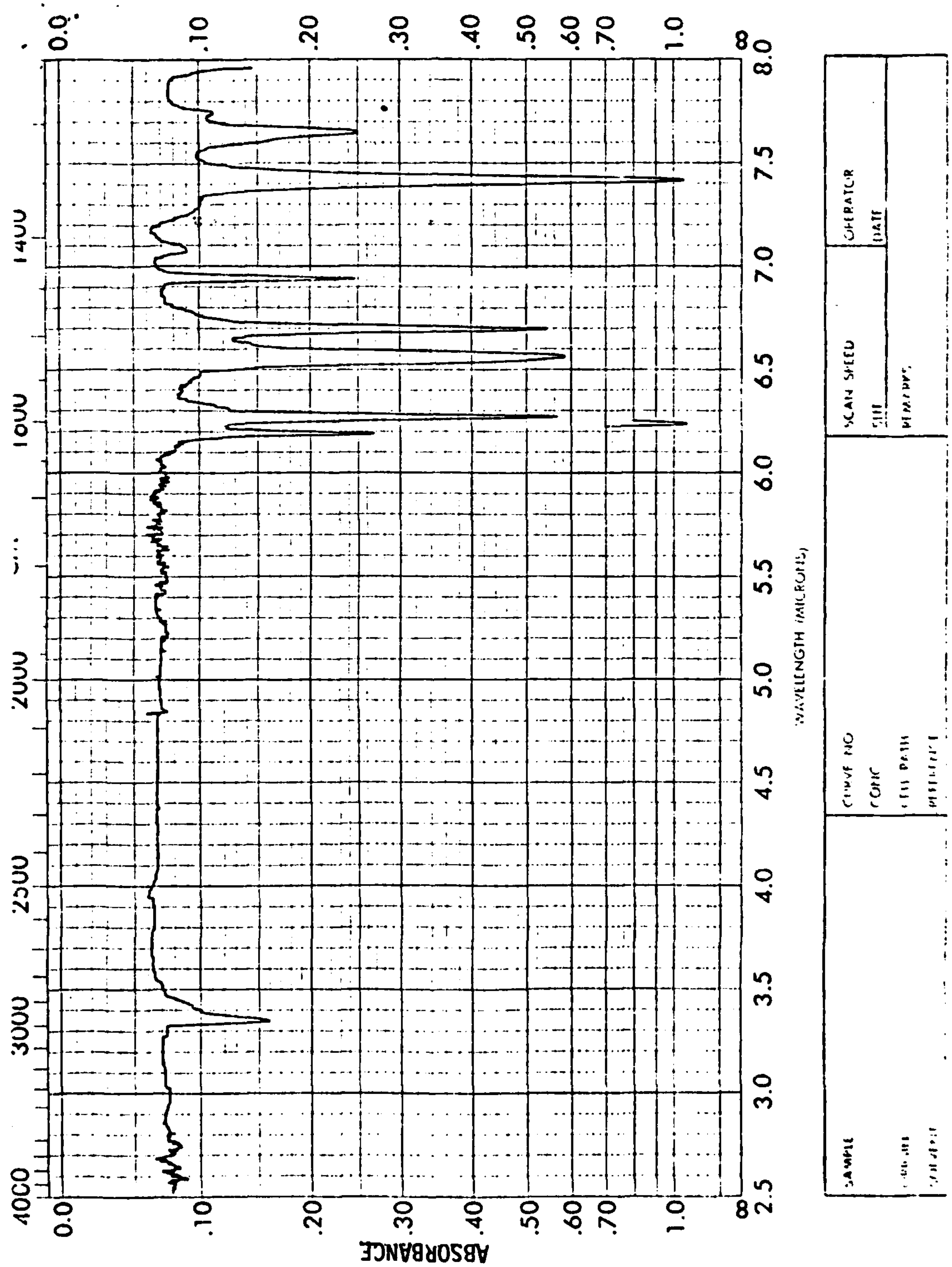
Thin Layer Chromotography: Analabs Uniplate, Silica Gel GF
Solvent: Ethyl Acetate
R_f = 0.51

Prepared by: M.A. Priest

Notebook No.: MP-07-29

Chemical Systems Research and Development

APPENDIX A (cont.)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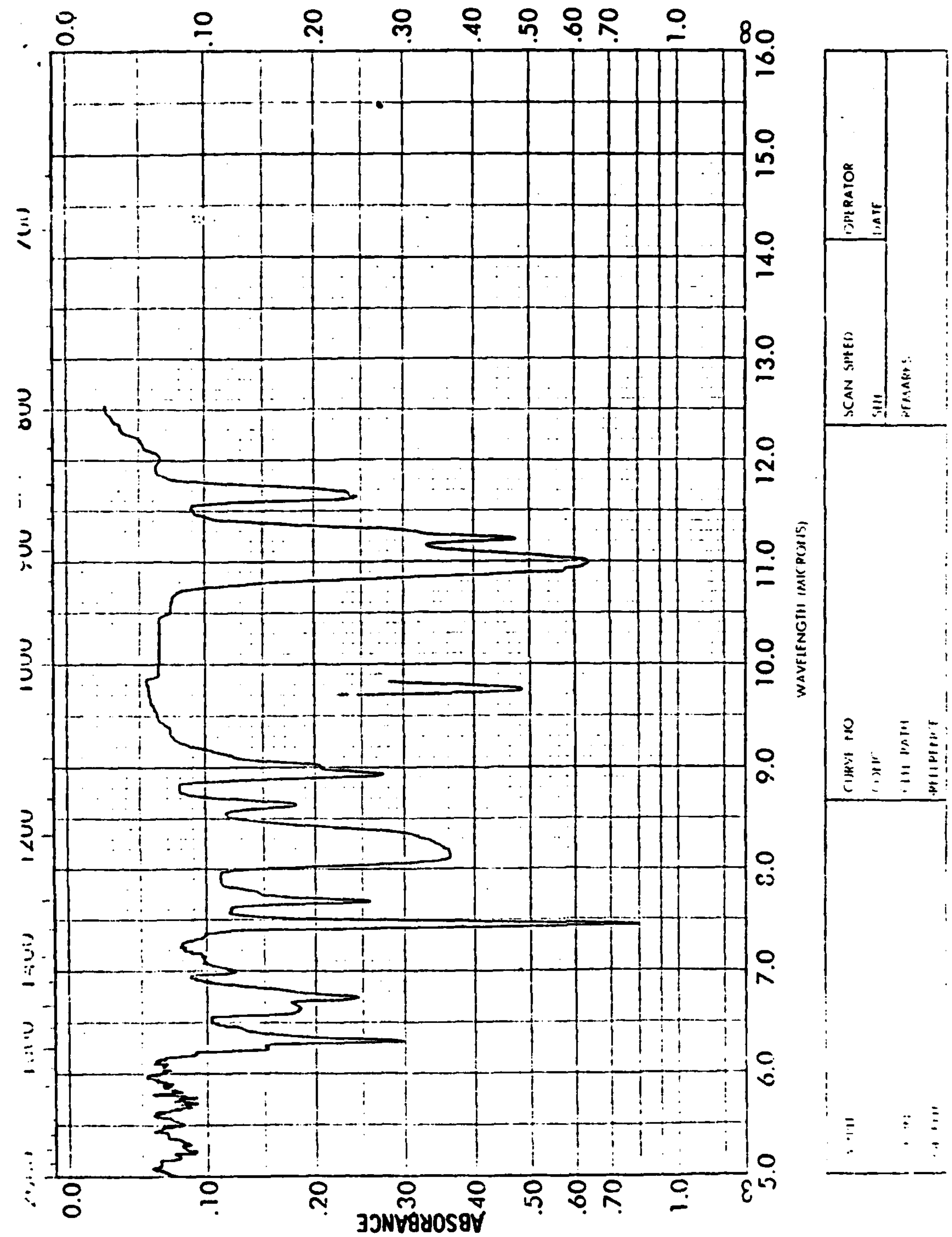
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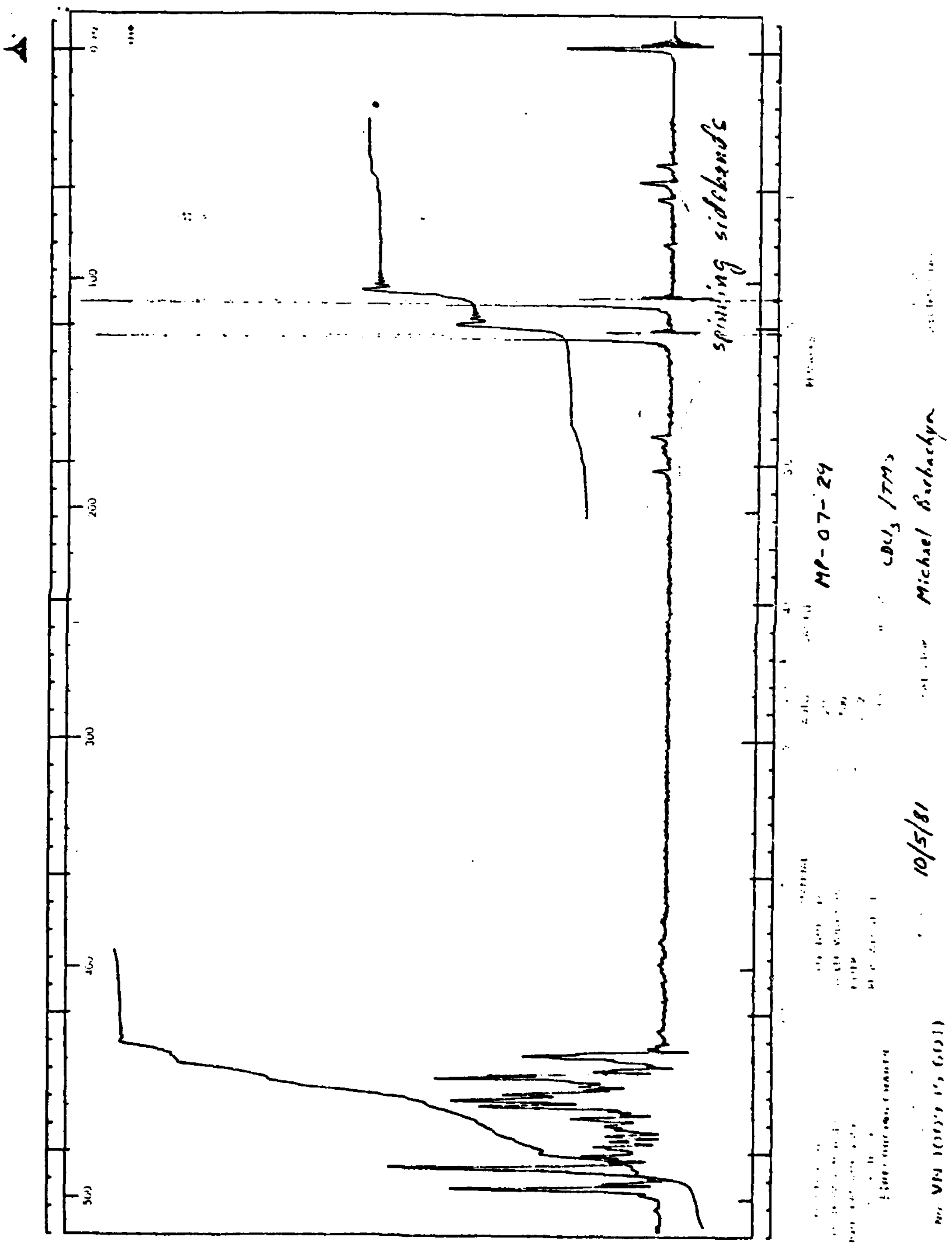

APPENDIX A (concluded)

Gross Pathology Summary and Interpretation of GLP Study
81033; LD_{50} 4-Nitrophenyl methyl Phenyl Phosphinate, Male
Sprague-Dawley Rats

The deaths of 6/7* male rats in Group 1 (60 mg/kg), 7/7 rats in Group 2 (45 mg/kg) and 2/7 rats in Group 3 (30 mg/kg) were attributed to the toxic efect of the tested compound. All deaths occurred between 22 minutes and 121 minutes following administration of the test compound by gastric intubation. None of the male rats in Group 4 (5 mg/kg), Group 5 (15 mg/kg) or Group 6 (controls) died prior to the scheduled termination of the study 14 days after administration of the test compound.

Gross changes attributable to the test compound were present in all of the rats that died. Lungs of these rats were mottled in appearance with dark red areas alternating with lighter pink areas. Lungs were wet and exuded bloody fluid from the cut surface. These changes were probably due to either congestion or hemorrhage or both. Livers were very dark red and contained much blood, changes typical of congestion. These lesions may be due to either direct effect of the test chemical on hepatic and pulmonary vasculature or secondary to compromised cardiac function.

Clear oily fluid was present around the nose and mouth of 6/7 rats in Group 1, 7/7 rats in Group 2 and 2/7 rats in Group 3. This change was present in all of the animals that died as a result of the test compound and may have been due to reflux of material administered by gastric intubation. It did not occur in any of the animals that survived.

The small intestine of some of the rats that died as result of gastric administration of the test material contained orange-pink semisolid material. The incidence of this change was: 3/7 in Group 1 and 3/7 in Group 2. This change did not occur in animals that survived for two weeks. It is probably a result of excess glandular secretion, possibly with slight mucosal hemorrhage. Some of these changes may have been exaggerated by autolysis.

Thymuses in 2/7 Group 1 rats that died and 1/7 rat in Group 2 had multiple petechial hemorrhages. This change is often observed in rodents that have been dead for several hours and is not considered an effect of the test material.

In summary, the gross pathologic efects, in addition to death, that most likely were due to a single dose gastric intubation with 4 Nitrophenyl methyl phenyl phosphinate, that were observed in male

*Number of rats affected/Number of rats in the group

APPENDIX B

Sprague-Dawley rats in this study were:

1. Congestion and/or hemorrhage in lungs and liver.

2. Possible hemorrhage and excess glandular secretion in the small intestine.

Necropsies revealed no test compound-related lesions in male Sprague-Dawley rats that were killed at the termination of the study.

PAUL W. MELLICK, DVM, PhD
Diplomate, ACVP
LTC, VC
Chief, Pathology Services Group
Division of Research Support

21 Jun 82

APPENDIX B (cont.)

Gross Pathology Summary and Interpretation of GLP Study 81-033; LD_{50} 4-nitrophenyl methyl Phenyl Phosphinate, Female Sprague-Dawley Rats

The deaths of 7/7* female rats in Group 1 (60 mg/kg), 7/7 rats in Group 2 (45 mg/kg), 6/7 rats in Group 3 (30 mg/kg), and 3/6 rats in Group 5 (15 mg/kg) were attributed to the toxic effect of the tested compound. The death of 1/7 rats in Group 4 (5 mg/kg) may have been due to the combined effects of the tested compound and pre-existing renal impairment. This animal had severe obstructive urolithiasis which caused bilateral hydronephrosis, pyelonephritis and cystitis. All deaths occurred between 25 minutes and 77 minutes following administration of the test compound by gastric intubation. None of the female rats in Group 6 (controls) died prior to the scheduled termination of the study 14 days after administration of the test compound.

Gross changes attributable to the test compound were present in all of the rats that died. Lungs of these animals were mottled in appearance with dark red areas alternating with lighter pink areas. The lungs were wet and exuded bloody fluid from the cut surface. These changes were probably due to either congestion or hemorrhage or both. Livers were very dark red and contained much blood, changes typical of congestion. These lesions may be due to either direct effect of the test chemical on hepatic and pulmonary vasculature or secondary to compromised cardiac function.

Clear oily fluid was found around the nose and mouth of 6/7 rats in Group 1, 6/7 rats in Group 2, 6/7 rats in Group 3, and 3/6 rats in Group 5. This observation was limited to animals that died as a result of the test compound and may have been due to reflux of material administered by gastric intubation.

The small intestine of some of the animals that died as a result of gastric administration of the test material contained orange-pink semisolid material. The incidence of this change by dosage group was: 5/7 in Group 1; 4/7 in Group 2; 2/7 in Group 3; 1/7 in Group 4; and 2/6 in Group 5. This change did not occur in animals that survived for two weeks. It probably is a result of excess glandular secretion, possibly with slight mucosal hemorrhage. Some of these changes may have been exaggerated by autolysis.

The thymuses of some animals that died had multifocal hemorrhages or diffuse congestion. The change occurred in 3/7 rats in Group 1; and 1/7 in Group 5. This change is frequently observed in rodents that have been dead for several hours and is not considered an effect of the test material. Other lesions observed in this group included a

*Number of rats affected/Number of rats in group

APPENDIX B (cont.)

possible congenital deformity of the sternum in 1 rat in Group 2, hydronephrosis in 1/7 rats in Group 1 and 1/6 rats in Group 5, a small hypoplastic thymus in 1 rat in Group 4 that also had severe obstructive urolithiasis, bilateral pyelonephritis and cystitis. These changes were considered to be incidental lesions that were unrelated to administration of the test compound.

In summary, the gross pathologic effects, in addition to death, that most likely were due to single dose gastric intubation with 4-Nitrophenyl methyl phenyl phosphinate that were observed in female Sprague-Dawley rats in this study were:

1. Congestion and/or hemorrhage in lung and liver.

2. Possible hemorrhge and excess glandular secretion in the small intestine.

Necropsies revealed no test compound-related lesions in the female Sprague-Dawley rats that were killed at the termination of the study.

PAUL W. MELLICK, DVM, PhD
Diplomate, ACVP
LTC, VC
Chief, Pathology Services Group
Division of Research Support

21 Jun 82

APPENDIX B (cont.)

GLP STUDY: LD_{50} 4-Nitrophenyl Methyl Phenyl Phosphinate - Male DATE: 25 Nov 81 TO 17 Dec 81

GROUP #	ANIMAL #	PATH ACC #
1	D8100733	31582
1	D8100736	31584
1	D8100740	31586
1	D8100746	31587
1	D8100749	31588
1	D8100761	31591
1	D8100729	31674
2	D8100722	31578
2	D8100731	31580
2	D8100732	31581
2	D8100739	31585
2	D8100757	31589
2	D8100760	31590
2	D8100762	31592
3	D8100730	31579
3	D8100735	31583
3	D8100727	31672
3	D8100742	31677
3	D8100750	31682
3	D8100753	31685
3	D8100759	31690
4	D8100724	31669
4	D8100734	31675
4	D8100744	31678
4	D8100745	31679
4	D8100747	31680
4	D8100752	31684
4	D8100756	31688

GROSS NECROPSY OBSERVATION

Group #1

Dosage level - 60 mg/kg.
Nose and mouth - Clear oily fluid; 5/7 (#'s 733, 736, 740, 749, & 761).
Lungs - Congestion or hemorrhage, mottled; 6/7 (#'s 733, 736, 740, 746, 749, & 761).
Thymus - Multiple petechial hemorrhage; 2/7 (#'s 746 & 761).
Liver - Diffuse dark red/probable congestion; 6/7 (#'s 733, 736, 740, 746, 749 & 761).
Small intestine - Orange/pink material; 3/7 (#'s 733, 740 & 761).
Nose and mouth - Yellow oily fluid; 1/7 (#746).
Post mortem autolysis - General, minimal; 6/7 (#'s 733, 736, 740, 746, 749 & 761).

Group #2

Dosage level - 45 mg/kg.
Nose and mouth - Clear oily fluid; 7/7.
Lungs - Congestion or hemorrhage, mottled; 7/9.
Thymus - Multiple petechial hemorrhage; 1/7 (#722).
Liver - Diffuse dark red/probable congestion; 7/7.
Small intestine - Orange/pink material; 3/7 (#'s 732, 757 & 760).
Post mortem autolysis - General, minimal: 7/7.

Group #3

Dosage level - 30 mg/kg.
Nose and mouth - Clear oily fluid; 2/7 (#'s 730 & 735).
Lungs - Congestion or hemorrhage, mottled; 2/7 (#'s 730 & 735).
Liver - Diffuse dark red/probable congestion; 2/7 (#'s 730 & 735).
Post mortem autolysis - General, minimal; 2/7 (#'s 730 & 735).

Group #4

Dosage level - 5 mg/kg.
No gross lesions recognized.

SUMMARY

APPENDIX B (cont.)

GLP STUDY: LD_{50} 4-Nitrophenvl Methvl Phenyl Phosphinate - Male (cont) DATE: 25 Nov 81 TO 17 Dec 81

GROUP #	ANIMAL #	PATH ACC #
5	D8100720	31667
5	D8100725	31670
5	D8100726	31671
5	D8100728	31673
5	D8100741	31676
5	D8100754	31686
5	D81007[illegible]	31691
6	D8100718	31665
6	D8100719	31666
6	D8100721	31668
6	D8100749	31681
6	D8100751	31683
6	D8100755	31687
6	D8100758	31689

GROSS NECROPSY OBSERVATION

Group #5

Dosage level - 15 mg/kg.
No gross lesions recognized.

Group #6

Dosage level - Vehicle Control.
No gross lesions recognized.

SUMMARY

APPENDIX B (cont.)

GLP STUDY: LD_{50} 4-Nitrophenyl Methyl Phenyl Phosphinate - Female DATE: 25 Nov 81 TO 17 Dec 81

GROUP #	ANIMAL #	PATH ACC #
1	D8100764	31593
1	D8100767	31594
1	D8100768	31595
1	D8100771	31597
1	D8100780	31604
1	D8100802	31613
1	D8100804	31614
2	D8100775	31599
2	D8100777	31601
2	D8100792	31607
2	D8100793	31608
2	D8100794	31609
2	D8100797	31610
2	D8100807	31616
3	D8100769	31596
3	D8100774	31598
3	D8100791	31606
3	D8100798	31611
3	D8100805	31615
3	D8100810	31617
3	D8100773	31696

GROSS NECROPSY OBSERVATION

Group #1

Dosage level - 60 mg/kg.
Nose and mouth - Clear oily fluid; 5/7 (#'s 764, 767, 780, 802, & 804).
Lungs - Congestion or hemorrhage, mottled; 7/7.
Thymus - Multiple petechial hemorrhage; 3/7 (#'s 764, 768, & 780).
Liver - Diffuse dark red/probable congestion; 7/7.
Small intestine - Orange/pink material; 5/7 (#'s 767, 768, 771, 780, & 802).
Nose and mouth - Yellow oily fluid; 1/7 (#768).
Post mortem autolysis - General, minimal; 7/7.

Group #2

Dosage level - 45 mg/kg.
Nose and mouth - Clear oily fluid; 6/7 (#'s 775, 777, 792, 793, 794, & 807).
Lungs - Congestion or hemorrhage, mottled; 7/7.
Liver - Diffuse dark red/probable congestion; 7/7.
Small intestine - Orange/pink material; 4/7 (#'s 775, 777, 793, & 797).
Post mortem autolysis - General, minimal, 7/7.
Sternum - Slightly depressed (possible congenital deformity); 1/7 (#797).
Kidney - Unilateral hydronephrosis; 1/7 (#777).

Group #3

Dosage level - 30 mg/kg.
Nose and mouth - Clear oily fluid; 6/7 (#'s 769, 774, 791, 798, 805, & 810).
Lungs - Congestion or hemorrhage, mottled; 6/7 (#'s 769, 774, 791, 798, 805, & 810).
Liver - Diffuse dark red/possible congestion; 6/7 (#'s 769, 774, 791, 798, 805, & 810).
Small intestine - Orange/pink material; 2/7 (#'s 774 & 791).
Post mortem autolysis - General, minimal; 6/7 (#'s 769, 774, 791, 798, 805, & 810).

SUMMARY

APPENDIX B (cont.)

GLP STUDY: LD_{50} 4-Nitrophenyl Methyl Phenyl Phosphinate - Female (cont) DATE: 25 Nov 81 TO 17 Dec 81

GROUP #	ANIMAL #	PATH ACC #
4	D8100778	31602
4	D8100765	31692
4	D8100770	31694
4	D8100786	31700
4	D8100787	31701
4	D8100790	31704
4	D8100796	31705
5	D8100779	31603
5	D8100781	31605
5	D8100801	31612
5	D8100788	31702
5	D8100799	31706
5	D8100803	31707
6	D8100766	31693
6	D8100772	31695
6	D8100783	31697
6	D8100784	31698
6	D8100785	31699
6	D8100789	31703
6	D8100809	31708

GROSS NECROPSY OBSERVATION

Group #4

Dosage level - 5 mg/kg.
Lungs - Congestion or hemorrhage, mottled; 1/7 (#778).
Liver - Diffuse dark red/probable congestion; 1/7 (#778).
Small intestine - Orange/pink material; 1/7 (#778).
Thymus - Very small; 1/7 (#778).
Kidneys - Left kidney is enlarged about 3 times normal size. There are numerous enlarged yellow areas visible through the capsular surface. The left ureter is greatly dilated. The right kidney is enlarged twice normal size. Right ureter is nearly normal, however. On cut surface the left kidney had a severely dilated renal pelvis filled with yellow creamy purulent material. The dilation was so severe that the cortex was reduced to a thin rim of tissue. The right kidney had a moderately thickened but otherwise normal cortex. There was a small focal accumulation of yellow creamy purulent material in the pelvis; 1/7 (#778).
Urinary bladder - The urinary bladder was dilated and distinctly yellow on the serosal surface. In the lumen there were ten yellow rough-surfaced calculi, the largest measuring 10 mm x 5 mm. Others were much smaller with the smallest approximately 2 mm diameter; 1/7 (#778).

Group #5

Dosage level - 15 mg/kg.
Nose and mouth - Clear oily fluid; 3/6 (#'s 779, 781, & 801).
Lungs - Congestion or hemorrhage, mottled; 3/6 (#'s 779, 781, & 801).
Thymus - Multiple petechial hemorrhage; 1/7 (#781).
Liver - Diffuse dark red/possible congestion; 3/6 (#'s 779, 781, & 801).
Small intestine - Orange/pink material; 2/6 (#'s 781 & 801).
Post mortem autolysis - General, minimal; 3/6 (#'s 779, 781, & 801).
Kidneys - Bilateral hydronephrosis; 1/6 (#788).

Group #6

Dosage level - Control.
No gross lesions recognized.

SUMMARY

APPENDIX B (concluded)

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